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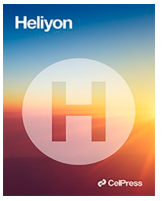

Research article

Evaluation of antinutrients, nutritional, and functional properties in sacha inchi (*Plukenetia volubilis* L*)* cake treated with hydrothermal processes

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A R T I C L E I N F O

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A B S T R A C T

Applying heat treatments using an autoclave and hot air sterilization can alter the proximal composition, technofunctional properties, and antinutrient content of Sacha inchi *(P. volubilis)* oil press cake. The autoclave and hot air treatments significantly reduced antinutrients compared to the control. The samples treated with autoclave and hot air sterilization exhibited a significant decrease in alkaloids, nitrates, tannins, saponins, and trypsin inhibitors compared to the control sample. However, the 20-min autoclave treatment did not significantly reduce the saponin antinutrients. Phytic acid significantly decreased in the 30-min hot air sample and autoclave 20-min/hot air treatments, respectively. On the other hand, the levels of antinutrients oxalate and thiocyanates did not significant difference between the control and hot air treatments. However, the autoclave treatment resulted in a significant reduction in oxalates. The study found that hydrotreatments at temperatures of 121 °C with humid heat - autoclave showed significant differences in protein content compared to the control sample, with content of 37.75 + 0.2 g/100g. Samples treated with an autoclave for 10, 20, and 30 min showed values of protein 53.19 + 0.28, 66.08 + 2.6, and 70.12 + 0.48 g/100g, respectively. Meanwhile, samples treated with dry heat showed significant differences with the sample treated for 10 min having a protein content of 60.21 + 6.80 g/100g. The techno-functional properties analyzed in the study demonstrated a significant decrease in hydrating properties such as water holding capacity (WHC), water retention capacity (WRC), and swelling capacity (SC) due to changes in the solubility of proteins for the two treatments and the oil holding capacity (OHC) property showed a significant increase. Finally, water's presence during hydrothermal treatments significantly reduces antinutrients, providing guidance for analyzing other study variables.

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1. Introduction

The Sacha Inchi plant *(Plukenetia volubilis* L.*)* also known as Inca Peanut, is a climbing plant that grows in tropical climates and is well-known for its oil-producing capabilities. Native to several South American countries, including Colombia, Bolivia, Ecuador, Brazil, Venezuela, and Suriname. Recently, its popularity has grown as it is recognized as a valuable source of plant-based oil and protein [1–3].

Sacha inchi *(P. volubilis)* seeds are highly valued for their oil, rich in unsaturated fatty acids and antioxidants [4–6]. The oil is extracted from the seeds by mechanical pressing, which is considered the most effective method to obtain oil free of contaminants [7–9]. In addition to oil, the seed extraction process also yields a byproduct called cake, which is high in protein with approximately 53–59 % of protein and has essential amino acids such as leucine, tyrosine, isoleucine, lysine and tryptophan [5,10–13]. However, the presence of antinutrients, such as tannins, phytic acid, saponins, hemagglutinins, and trypsin inhibitors, in Sacha inchi *(P. volubilis)* seeds and cake can limit their use in the food industry [3,14]. Therefore, evaluating different methods to eliminate these antinutrients and facilitate their use in various food products is essential.

In recent years, alternative protein sources in human nutrition have become increasingly important due to the growing demand for sustainable and healthy food products. One such source of protein is Sacha inchi *(P. volubilis)*, a plant native to the Amazon rainforest. The use of Sacha inchi *(P. volubilis)* flour in various food products has been studied and has shown potential as a replacement for soy and wheat flour [15]. Before Sacha inchi *(P. volubilis)* flour can be widely used as a raw material in human nutrition, it is important to ascertain the presence of antinutrients in Sacha inchi *(P. volubilis)* cake, similar to other protein sources such as soy *(G.max)*, chocho *(L. mutabilis), and quinoa (Ch. Quinoa)* but also contain antinutrients such as saponins, phytic acid, alkaloids, trypsin inhibitors, tannins, nitrates, and ureasic activity [16–18]. Recent studies have focused on Sacha inchi seed and cake, exploring various thermal treatments that deactivate antinutrients, alter the oil's characteristics, and reduce specific heat labile antinutrients [3,19]. However, the effect of these treatments on some of the antinutrients found in Sacha inchi cake and the difference in the impact of water on the decrease in antinutrients are areas of research yet to be explored. This study will evaluate the impact of a hydrothermal treatment as an autoclave (AC) and hot air (HA) on the antinutrient content and the resulting changes in the flour's technological and nutritional properties. Furthermore, the discussion will encompass the examination of antinutrients post-treatment.

2. Materials and methods

2.1. Sample preparation

The Sacha inchi *(P. volubilis)* defatted cake was provided by the industrial plant Agroindustrias G2 S.A., located in the Province of Pichincha, Ecuador. The raw material was sourced from the city of Muisne, which is situated in the province of Esmeraldas, Ecuador. This cake is a byproduct of oil extraction by cold pressing and has less than 10 % humidity.

According to Suwanangul et al.[19]samples were prepared in 6 suspensions at 10 % (w/v), with 500 g of each sample in filtered water and triplicate for heat treatment. The prepared sample suspensions were exposed to hot air for 10, 20, and 30 min and autoclaved with high-pressure saturated steam at 121 °C for 10, 20, and 30 min. The control was dried in hot air at 50 °C for 22 h. Finally, the dried samples were pulverized for each test and were conducted in triplicate (Fig. 1).

The experiment used a BIOBASE vertical autoclave model BKQ-B100II to apply high-pressure saturated steam at 1 bar of pressure for 10, 20, and 30 min, depending on the treatment. Subsequently, the samples were dried in a forced air oven at 50 °C.

For hot air treatments, a Memmer model SN75 equipment with natural convection and electronically adjustable air extraction was

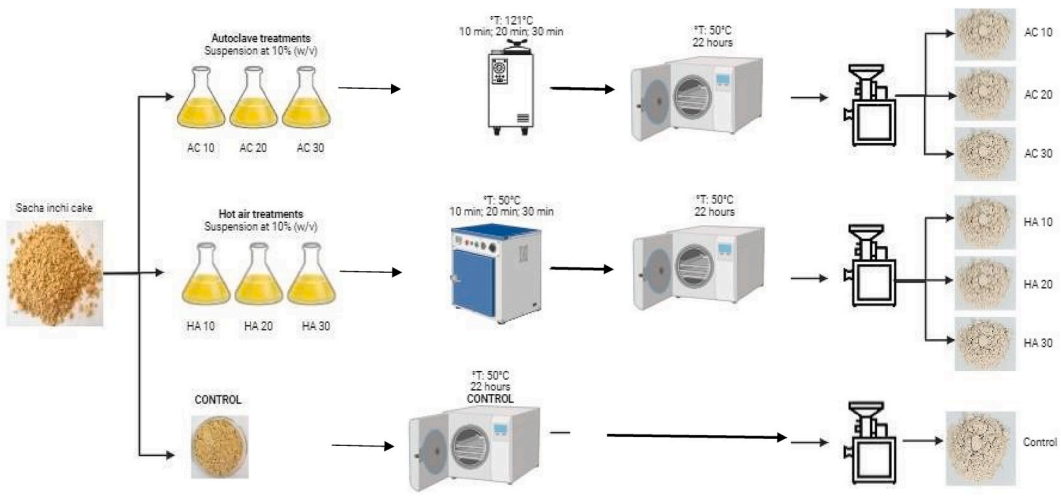

Fig. 1. Treatments conducted on samples of Sacha inchi cake.

used, set to a sterilization temperature of 121 °C for 10, 20, and 30 min.

2.2. Antinutritional factors

2.2.1. Nitrates

Nitrate quantification was carried out using the method reported by Cataldo et al. [20]. Before analysis, samples were homogenized and filtered in a 0.34 M K_2SO_4 solution. The filtrate (0.5 mL) was then mixed with 5 % salicylic acid and NaOH (4N), and the absorbance at 410 nm was measured using a U.V.–Visible spectrophotometer (Thermo Fisher Scientific 201 Evolution, Madison, WI USA). The nitrate content was expressed in milligrams per kilogram on a dry weight basis.

2.2.2. Tannins

Tannin content was determined using Folin-Denis reagent [21] and tannic acid as a standard. Absorbance was measured at 680 nm and expressed as mg per 100 g (dry weight basis).

2.2.3. Quinolizidine alkaloids (QAs)

The total alkaloid content of the sample was determined using a modified version of von Baer's method for titration. Firstly, 5 mL of 0.01 N sulfuric acid and two drops of methyl red were added to the concentrated chloroform extract. Then, the excess acid was titrated with 0.01 N NaOH. To calculate the amount of alkaloid in the sample, it was determined that 1 mL of 0.01 N H_2SO_4 was equivalent to 2.48 mg of lupanine [22].

2.2.4. Phytic acid

The Megazyme method was used to measure Phytic acid [23]. This method measures the total "available phosphorus" present in the sample. Firstly, an acid extraction of inositol phosphate is done, followed by treatment with phytate, which is specifically designed for phytic acid (IP6), as well as the lower forms of inositol phosphate (IP2, IP3, IP4, IP5). After that, the sample is treated with alkaline phosphatase, which ultimately ensures that phytate is released from myoinositol phosphate (IP1), which is quite resistant to the action of phytate. The total amount of phosphate released is measured using a modified colorimetric method on a Thermo Scientific 201 Evolution spectrophotometer at a wavelength of 655 nm. This results in the quantity of phosphorus in grams per 100 g of sample.

2.2.5. Trypsin inhibitors (T.I.)

The measurement of trypsin inhibitor activity was performed using the AOCS method (2009) [24]. To extract T.I., 1.00 g of defatted Sacha inchi cake was mixed with 50.0 mL of 0.01 M NaOH and agitated for 3 h at room temperature. The resulting suspension was then subjected to a final centrifugation step for 10 min at 10,000×*g* to separate the supernatant (Sacha inchi flour extract) for the T.I. assay.

2.2.6. Total saponins

The total saponins content was measured using a spectrophotometer, following the method described by Medina-Meza et al. [25]. To obtain the extract, 1 g of defatted flour was mixed with 25 mL of 80 % methanol and shaken for 1 h. The mixture was then filtered under vacuum and made up to 25 mL with 80 % methanol. To prepare the reagent mixture, glacial acetic acid and sulfuric acid were mixed in a 1:1 ratio. 0.50 mL of the extract was mixed with 1 ml of the reagent mixture, and then stirred in a 60 °C water bath for 30 min and cooled. The Thermo Scientific Evolution 201 spectrophotometer was used to measure the absorbance of the samples at a wavelength of 527 nm. The absorbance readings were then compared to a standard curve prepared from total saponin.

2.2.7. Oxalates

The extract is prepared using 0.25 N HCl as the extraction solution and $KMnO_4$ as an indicator, following the method described by Naik et al. [26].

2.2.8. Glucosinolates

The process utilized to extract and determine glucosinolates followed the standard *ISO 9167:2019* [27]. Initially, 500 mg of dried sample was mixed with 5 mL of boiling 70 % methanol and kept in a 70 °C bath for 5 min, stirring occasionally. Following this, the mixture was centrifuged at 9000 rpm for 5 min, and the supernatant was separated, after which the process was repeated on the solid. The two supernatants were combined and dried using a rotary evaporator at 40 °C, and the solids were then reconstituted with 2 mL of distilled water. Next, 0.2 mL of the extract was mixed with 0.2 mL of 2M NaOH and incubated at room temperature for 30 min. The mixture was centrifuged at 4000 g for 10 min after adding 31 μL of concentrated HCl. For spectrophotometric determination, 100 μL of the supernatant was combined with 100 μL of a 2 mM ferricyanide solution in 0.2 M phosphate buffer pH 7.0. Lastly, the absorbance of the solution was measured at 420 μm using 0.2 M phosphate buffer pH 7.0 as a blank. The calibration curve was constructed using a 5.60×10^{-3} M sinigrin standard.

2.3. Proximal composition determination

The proximate analysis of the chemical composition was carried out following the methodologies of the Official Association of Analytical Chemistry (2005) for moisture, ash, ether extract, and protein (AOAC 925.10 – AOAC 923.03 – AOAC 920.85 – AOAC 2011.11) [28]. Considering the lack of fiber value in the composition of the samples, the method established by FAO was used to

calculate the amount of apparent carbohydrates and caloric value by difference [29].

2.3.1. Amino acid analysis

To determine the amino acid content of Sacha inchi cake and flour, the samples are dissolved in a 0.1 M potassium phosphate buffer solution with a pH of 8. The protein in the solution is then oxidized using a solution of hypochlorite or peracetic acid in a ratio of ¼ (v/v) and incubated for 10 min at 30 °C, following the methodology used by Kerkaert [30]. After oxidation, Trichloroacetic acid (TCA) separates the protein of 100 mg of the sample. The sample is then redissolved in 5 ml of 0.1M phosphate buffer (pH 8) with 50 μL of 10 M sodium hydroxide. Finally, the sample undergoes acid and basic hydrolysis to obtain amino acids [31]. The sample is mixed with o-phthalaldehyde (OPA) and homogenized to derivate amino acids [32]. The mixture is then injected into a High-Performance Liquid Chromatography coupled with a fluorescence detector (HPLC-FLD, Agilent Technologies, Switzerland) under the following conditions: The derivatized amino acids are separated on a Zorbax Eclipse AAA Rapid Resolution column (4.6 × 150mm, 5 μm, Agilent Technologies). The wavelengths used for solvent A (45 % methanol, 45 % acetonitrile, and 10 % water) and solvent B (45 mM NaH_2PO_4 3H2O, 0.02 % NaN_3, pH7.8) are 340–450 nm and 266–305 nm, respectively, according to Agilent application note [33].

2.4. Functional properties. (S.C., WHC, WRC, OHC, A.E. and E.E.)

2.4.1. Swelling capacity (S.C.)

The swelling capacity was determined using the methods of Raghavendra et al. and Robertson and Roertson et al. [34,35]. A sample of approximately 0.2 g was weighed and placed in a conical tube to conduct the test. Then, 10 mL of filtered water was added to the sample, and the mixture was allowed to hydrate for 18 h at a 25 °C. After 18 h, the final volume of the powder was measured.

2.4.2. Water absorption capacity (WHC)

The water absorption capacity was determined according to the method used by Raghavendra et al. and Robertson et al. [34,35]. A sample (≈0.2 g) was weighed, the sample was placed in a conical tube, and 10 mL of filtered water was added. The samples were left to hydrate for 18 h at 25 °C. After that, the supernatant was removed, and the hydrated residue was weighed. The residue was then freeze-dried, and its weight was recorded.

2.4.3. Water retention capacity (WRC)

The water retention capacity was determined according to the method used by de Escalada Pla et al. and Raghavendra et al. [34,36]. A sample (≈1 g) was weighed, then the sample was placed in a conical tube, and 10 mL of filtered water was added. The samples were hydrated for 18 h at 25 °C and then centrifuged (Centrifuge TGL 16 Yingtai Instrument®) at 2000 rpm for 30 min. The supernatant was separated, and the residue (remaining wet solid) was weighed. The residue is freeze-dried, and its weight is recorded.

2.4.4. The oil holding capacity (OHC)

The Oil Holding Capacity (OHC) was measured following the method used by Garau et al. [37]. To do this, samples were mixed with sunflower oil, left overnight at room temperature, and then centrifuged using the Centrifuge 5430 R Eppendorf® at 1500×*g* for 5 min. After centrifugation, the supernatant was decanted, and the sample was weighed. The OHC was calculated based on the weight gain and expressed as the amount of oil absorbed per gram of dry sample.

Table 1
Content of antinutrient compounds in sacha inchi (*P. volubilis*) flours with two thermal treatments (dry weight basis).

	Alcaloids (% BS)	Oxalate % (P/P)	Nitrates (mg/100g)	Tannin % (P/P)	Thiocyanates (mg/100g)	Saponins (mg/100g)	Phytic acid (g/100g)	Trypsin inhibitor (TI mg/g)
Control	1.610 ± 0.000^{f}	0.450 ± 0.000^{d}	2.280 ± 0.000^{e}	0.216 ± 0.000^{e}	0.580 ± 0.000^{ab}	13.600 ± 0.000^{d}	2.640 ± 0.000^{d}	5.090 + 0.000^{e}
AC 10	0.320 ± 0.000^{d}	0.150 ± 0.014^{a}	1.340 ± 0.014^{c}	0.155 ± 0.001^{c}	0.625 ± 0.007^{d}	11.400 ± 0.000^{b}	3.105 ± 0.001^{e}	1.310 + 0.014^{a}
AC 20	0.115 ± 0.007^{a}	0.185 ± 0.007^{b}	1.470 ± 0.014^{b}	0.105 ± 0.006^{a}	0.610 ± 0.000^{cd}	16.700 ± 0.141^{e}	1.780 ± 0.028^{a}	1.615 + 0.049^{c}
AC 30	0.145 ± 0.007^{b}	0.315 ± 0.007^{c}	1.080 ± 0.028^{a}	0.102 ± 0.003^{a}	0.625 ± 0.007^{d}	7.750 ± 0.071^{a}	3.220 ± 0.028^{f}	1.440 + 0.014^{b}
HA 10	0.810 ± 0.014^{e}	0.455 ± 0.007^{d}	1.140 ± 0.000^{a}	0.142 ± 0.001^{b}	0.595 ± 0.007^{bc}	12.850 ± 0.071^{c}	2.435 ± 0.021^{c}	1.460 + 0.057^{b}
HA 20	0.285 ± 0.007^{c}	0.455 ± 0.007^{d}	1.335 ± 0.021^{c}	0.190 ± 0.001^{d}	0.565 ± 0.007^{a}	7.950 ± 0.071^{a}	2.380 ± 0.000^{c}	1.515 + 0.021^{bc}
HA 30	0.285 ± 0.007^{c}	0.425 ± 0.007^{d}	1.270 ± 0.000^{b}	0.136 ± 0.001^{b}	0.570 ± 0.000^{a}	8.100 ± 0.141^{a}	1.950 ± 0.014^{b}	1.880 + 0.028^{d}
p-value								
time	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000
treatment	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
time*treatment	0.000	0.000	0.000	0.000	0.027	0.000	0.000	0.000

Values with different letters in the same column are significantly different ($p < 0.05$) ($n = 2$.

2.5. Statistical analysis

The antinutrients analyses was conducted by duplicate and the proximate, technofuntional properties and amino acid by triplicate. To analyze the statistical data of the proximate, technofuntional properties, amino acid content and antinutrients, two tests were conducted using Minitab® 19.20201 (64bit) software (Minitab Inc. Pennsylvania, USA) for statistical analysis. Firstly, the one-way ANOVA test was performed using the Tukey method to determine any significant differences between the experiments, including the control sample. Secondly, the two-way ANOVA test was conducted to identify the influence of each factor (time and treatment) and their combination of them (p-value).

3. Results and discussion

3.1. Antinutrients

3.1.1. Alkaloids, oxalates, nitrates, tannins, thiocyanates, saponins

The results of the quantification of alkaloids, oxalates, nitrates, tannins, thiocyanates and saponins, as well as the significant statistical differences ($p < 0.05$) between treatments of Sacha inchi (*P. volubilis)* cake pressing are presented in Table 1. The content of alkaloids varied with heat treatment applied to the pressing cake of Sacha inchi (P. volubilis) ($p < 0.05$). The control presented the highest content (1.61 g/100 g) and was lower than that reported for L. mutabilis (3.30–3.10 %) [38]. Alkaloid contents of 3.8 %, 2.74 % and 1.6 % have been reported for *L. albus, L. campestris and L. angustifulius*, respectively [38,39]. The treatment with AC at 20 min reduced alkaloids by 92.85 % relative to the pressing cake. Notably, the lowest contents were recorded in the following treatments: 0.115 % (AC 20 min) and 0.145 % (AC 30 min), levels that are not only significantly reduced but also considered safe for human consumption according to the health authorities of the UK, France, Australia and New Zeland [40].

The results obtained in the alkaloid content in the autoclave treatment are lower than those of the hot air treatment due to the solubility of the alkaloids in water and the heat that contributed to the extraction and inactivation of the alkaloids [41].

Oxalates can cause harm to the kidneys, leading to kidney damage [42]. The results showed that autoclaving for 10 min reduced the oxalate content by 66.67 %, and autoclaving for 20 min reduced it by 60 %, mainly due to the reduction of soluble oxalates [43]. However, hot air sterilization did not show a significant difference from the control sample, as there was no reduction in soluble oxalates, which may be predominant in Sacha inchi cake.

The nitrate values in Sacha inchi (*P. volubilis)* pressing cake were lower than those reported for other vegetables like spinach and leaf lettuce [44]. The amount of nitrate in the cake varied depending on the thermal process applied, with the lowest concentration being found in AC 30 min (1.08 mg/100 g). The nitrate content was reduced by 52.63 % and 50 % in AC 30 min and HAS 10, respectively, due to nitrate water solubility and the effects of heat. The nitrate concentrations in the cake were within the permissible limits set by the regulations of some European countries [45].

Antinutritional compounds, such as tannins, can affect mineral and protein utilization, leading to growth depression by decreasing the digestibility of protein and carbohydrates [46,47]. The concentration of tannins varies depending on the thermal treatment applied and time ($p < 0.05$). The highest concentration of tannins (0.216 g/100 g) was found in the control treatment, which is higher than soy (45 mg/100 g) and lower than raw *Lupinus termis* seeds (753 mg/100 g) [46]. The tannin contents shown in Table 1 significantly decreased by 51.39 %–52.78 % after the autocalve process (AC) for 20 or 30 min. High temperatures break down the tannin-protein complex, inducing the leaching of tannins in the cooking liquid, thus increasing the digestibility and palatability of the pressing cake [46]. Similar changes were seen in Sacha inchi when it was processed using autoclaving and extrusion at 121 °C, resulting in 92 % and 31 % reductions, respectively [19]. Additionally, in *L. campestris*, the tannin content was reduced by 77 % through alkaline aqueous treatment and by 70 % through regularaqueous treatment during the debittering under alkaline conditions [39].

Table 1 shows that Sacha inchi (*P. volubilis)* pressing cake contains Thiocyanates, wich can act as goitrogenic substances and prevent the formation of thyroid hormones. Thiocyanates can be deactivated at temperatures above 150 °C and pH of 2, wich explains the minimal reduction and increase of these antinutrients in the samples [48]. According to the US Department of Health and Human Services (ATSDR) [49], the level of thiocyanates found in the pressing cake of P. volubilis are safe for consumption. Thiocyanate is also found in many other foods such as plants, dairy products, and meat, and it is eliminated from the body quickly in urine or breath [49].

Saponins are heat-resistant, water-soluble secondary metabolites in high concentrations in *P. volubilis* [50,51]. However, due to their bitter taste, it was necessary to reduce their concentration by applying thermal treatments such as AC for 30 min, which resulted in a significant decrease (43 %), along with HA for 20 and 30 min.

3.1.2. Trypsin inhibitors and phytic acid

The levels of trypsin inhibitors in Sacha inchi seeds were significantly reduced throught roasting at temperatures between 80 and 160 °C as part of the thermal processes [3]. On average, the AC 20 min treatment had 1.31 TIU mg/g, which is similar to the values seen in other species such as *L. exaltatus* (1.37 TIU mg/g) and *L. reflexus* (2.05 TIU mg/g) [52]. The application of AC 20 min treatment resulted in a decrease of 74.26 % in TIUs during the cake pressing of Sacha inchi (*P. volubilis)*.

Phytic acid is often considered an antinutrient since it can bind minerals, proteins, and starches, decreasingin their bioavailability [53].

Multiple comparison tests showed the thermal process had significant effects on phytic acid content in Sacha inchi (*P. volubilis)* ($p < 0.05$). AC 20 min treatmen had 1.78 g phytic acid/100 g, which is higher to values that have been reported for other beans (0.46 g/100 g) but similar compared with other grains and legumes, such as *L. exaltatus* (1.17 g/100 g), *L. albus* (1.42 g/100 g), *L. angostifolius*

(1.45 g/100 g) [38,52] and soy (1.27 g/100 g) [54,55]. The initial concentration of 2.64 mg phytic acid/100 g (dry weight basis) was reduced to 1.78 mg phytic acid/100 g (dry weight basis) after the thermal process, which represents a reduction of 32.57 %. These findings are consistent with previous studies that reported a reduction in phytic acid in soaked and hydrothermally processed *Bauhinia purpurea* and *P. volubilis* [3,19,56].

3.2. Proximate composition

Table 2 summarizes the proximate composition of Sacha inchi (*P. volubilis)* cake (control) and the autoclave and hot air sterilization treatments. Table 2 shows that time is the main factor affecting the moisture, ash, and ethereal extract results, while the protein content is significantly influenced by the treatment and the combination of time and treatment factors. The highest protein content was found in the flour treated with an autoclave. The protein content of flour treated for 10, 20, and 30 min treated with an autoclave was significantly higher than the untreated flour, while the 20 and 30 min treatment with HA had no difference in protein content as the untreated flour - control. This indicates that treatments up to 30 min can be applied without reducing the original protein content. The rise in protein content in the 10- and 20-min. treatments is probably a result of the nutrient becoming more concentrated after the reduction of antinutrients. Additionally, the autoclave treatment could also impact the protein increase by extracting soluble proteins, and causing the formation of new proteins due to an aggregation process after the denaturation of protein [57]. However, cooking at temperatures higher than 100 °C and prolonged cooking times can cause protein denaturation, leading to a decrease in protein content [58–60]. The 10-min treatments have the highest total carbohydrate values compared to the 20- and 30-min treatments. The flour treated with AC 20, AC 30, HA 20, and HA 30 has the highest protein and the lowest carbohydrate content. There is no difference in the ethereal extract and caloric values between the treatments.

3.2.1. Amino acid profile

Table 3 shows that the treatments applied do not affect most amino acids ($p > 0.05$). The exceptions are aspartic acid and isoleucine, which are affected by the type of treatment, reflecting a significant decrease when sacha inchi cake is subjected to the HA treatment. On the other hand, the combination of the factors of time and type of treatment strongly affect glycine, isoleucine and lysine.

The amino acid content in Sacha inchi cake and flours treated with autoclave and hot air is lower than in untreated Sacha inchi cake, as shown by Hamaker, Ruiz, Rawdkued, and Hurtado Ordoñez [10,11,14,61]. However, the thermal treatments used in these processes can cause protein denaturation, leading to the formation of protein aggregates. This is due to the high temperatures and pressure exerted during the denaturation process, which can form new covalent and non-covalent bonds that lead to protein aggregation [57,62].

3.3. Functional properties

The applications of Sacha inchi flour in the food industry are determined by its functional properties. The research carried out in this study used the treatments listed in Table 4, all of which were conducted at a temperature of 121 °C. According to research conducted by Saio et al. [63], an inversely proportional relationship exists between temperature and the hydration properties of soy flour with high amounts of protein. This is due to the partial extraction of soluble proteins, which results in lower nitrogen solubility.

Table 2
Proximate analyses of Sacha inchi control flour (with no treatment) and Sacha inchi flour with treatments, expressed in g/100 g of product dry basis.

Factor	Moisture (g/100g)	Ash (g/100g)	Protein (N × 6.25) (g/100 g)	Total carbohydrates by difference (g/100 g db)	Ethereal extract (g/100 g db)	Caloric Value (Kcal)
Control	4.415 ± 0.092^{bc}	6.215 ± 0.021^{a}	37.745 ± 0.276^{d}	46.00 ± 0.00^{a}	5.945 ± 0.332^{a}	387.00 ± 1.414^{ab}
AC 10	4.953 ± 0.149^{a}	5.185 ± 0.044^{c}	53.188 ± 0.279^{c}	29.250 ± 2.500^{b}	7.505 ± 2.198^{a}	397.00 ± 11.020^{ab}
AC 20	4.523 ± 0.177^{b}	5.640 ± 0.076^{b}	66.078 ± 2.626^{ab}	15.00 ± 3.559^{cd}	8.633 ± 1.344^{a}	402.50 ± 7,050^{a}
AC 30	4.185 ± 0.099^{c}	5.758 ± 0.068^{ab}	70.120 ± 0.484^{a}	11.500 ± 2.380^{d}	8.400 ± 2.491^{a}	402.250 ± 13.280^{a}
HA 10	5.103 ± 0.079^{a}	5.673 ± 0.421^{b}	60.213 ± 6.803^{bc}	23.750 ± 5.965^{bc}	4.997 ± 1.508^{a}	380.750 ± 7.500^{b}
HA 20	4.492 ± 0.117^{bc}	5.545 ± 0.041^{bc}	33.560 ± 1.223^{d}	49.250 + 2.217^{a}	7.498 ± 1.346^{a}	397.500 ± 7.00^{ab}
HA 30	5.027 ± 0.208^{a}	5.357 ± 0.108^{bc}	40.687 ± 5.544^{d}	43.250 ± 4.99^{a}	5.400 ± 1,316^{a}	385.25 ± 5.970^{ab}
p-value						
Time	0.001	0.200	0.073	0.099	0.474	0.363
Treatment	0.002	0.981	0.000	0.000	0.103	0.073
Time*treatment	0.003	0.005	0.000	0.000	0.798	0.664

Values with different letters in the same column are significantly different ($p < 0.05$) ($n = 2$).
db means dry basis.

Table 3
Amino acid content in an autoclave- and hot air-treated sacha inchi flour (g/100 g protein).

Factor	Leucine	Isoleucine	Phenylalanine	Histidine	Lysine	Threonine	Tryptophan	Valine	Metionine
Control	0.125 ± 0.000^{a}	0.008 + 0.000^{ab}	0.064 ± 0.000^{a}	0.103 ± 0.000^{a}	0.135 ± 0.000^{ab}	0.087 ± 0.000^{a}	0.053 ± 0.000^{a}	0.034 ± 0.000^{a}	0.016 ± 0.000^{a}
AC 10	0.152 ± 0.003^{a}	0.009 + 0.000^{ab}	0.099 ± 0.001^{a}	0.161 ± 0.001^{a}	0.097 ± 0.002^{b}	0.039 ± 0.000^{a}	0.151 ± 0.001^{a}	0.037 ± 0.001^{a}	0.023 ± 0.000^{a}
AC 20	0.240 ± 0.002^{a}	0.016 + 0.002^{a}	0.167 ± 0.012^{a}	0.253 ± 0.000^{a}	0.154 ± 0.011^{a}	0.063 ± 0.000^{a}	0.240 ± 0.002^{a}	0.057 ± 0.000^{a}	0.036 ± 0.000^{a}
AC 30	0.203 ± 0.002^{a}	0.015 + 0.000^{a}	0.132 ± 0.002^{a}	0.211 ± 0.000^{a}	0.134 ± 0.002^{ab}	0.054 ± 0.000^{a}	0.200 ± 0.002^{a}	0.049 ± 0.000^{a}	0.030 ± 0.000^{a}
HA 10	0.175 ± 0.060^{a}	0.013 + 0.000^{ab}	0.129 ± 0.021^{a}	0.162 ± 0.094^{a}	0.151 ± 0.019^{a}	0.066 ± 0.015^{a}	0.138 ± 0.112^{a}	0.041 ± 0.017^{a}	0.025 ± 0.010^{a}
HA 20	0.163 ± 0.059^{a}	0.005 + 0.000^{b}	0.127 ± 0.038^{a}	0.166 ± 0.077^{a}	0.129 ± 0.006^{ab}	0.069 ± 0.019^{a}	0.133 ± 0.109^{a}	0.040 ± 0.019^{a}	0.024 ± 0.008^{a}
HA 30	0.166 ± 0.059^{a}	0.005 + 0.000^{b}	0.134 ± 0.032^{a}	0.166 ± 0.084^{a}	0.119 ± 0.019^{ab}	0.069 ± 0.019^{a}	0.135 ± 0.109^{a}	0.038 ± 0.017^{a}	0.024 ± 0.008^{a}
p-value									
Time	0.480	0.533	0.187	0.558	0.173	0.388	0.760	0.596	0.404
Treatment	0.250	0.007	0.851	0.251	0.508	0.068	0.216	0.321	0.186
Time*treatment	0.306	0.010	0.167	0.608	0.006	0.540	0.708	0.499	0.279

Factor	Alanine	Tyrosine	Aspartic acid	Glutamic acid	Arginine	Glycine	Serine
Control	0.138 ± 0.000^{a}	0.077 ± 0.000^{a}	0.162 ± 0.000^{ab}	0.241 ± 0.000^{a}	0.119 ± 0.000^{a}	14.540 ± 0.000^{ab}	0.164 ± 0.000^{a}
AC 10	0.162 ± 0.000^{a}	0.093 ± 0.001^{a}	0.181 ± 0.003^{ab}	0.257 ± 0.001^{a}	0.140 ± 0.001^{a}	9.444 ± 0.943^{b}	0.210 ± 0.001^{a}
AC 20	0.253 ± 0.004^{a}	0.146 ± 0.000^{a}	0.285 ± 0.002^{a}	0.402 ± 0.004^{a}	0.221 ± 0.000^{a}	18.942 ± 0.518^{a}	0.332 ± 0.002^{a}
AC 30	0.218 ± 0.002^{a}	0.119 ± 0.002^{a}	0.232 ± 0.002^{ab}	0.331 ± 0.002^{a}	0.188 ± 0.002^{a}	12.773 ± 1.413^{ab}	0.280 ± 0.000^{a}
HA 10	0.189 ± 0.062^{a}	0.109 ± 0.034^{a}	0.137 ± 0.042^{b}	0.302 ± 0.097^{a}	0.159 ± 0.056^{a}	14.773 ± 0.127^{ab}	0.238 ± 0.086^{a}
HA 20	0.119 ± 0.015^{a}	0.103 ± 0.034^{a}	0.118 ± 0.036^{b}	0.289 ± 0.092^{a}	0.154 ± 0.056^{a}	13.865 ± 0.436^{ab}	0.225 ± 0.086^{a}
HA 30	0.178 ± 0.067^{a}	0.105 ± 0.032^{a}	0.208 ± 0.066^{ab}	0.289 ± 0.092^{b}	0.152 ± 0.058^{b}	17.960 ± 4.280^{a}	0.225 ± 0.090^{a}
p-value							
Time	0.728	0.411	0.111	0.441	0.464	0.043	0.499
Treatment	0.067	0.344	0.008	0.344	0.275	0.149	0.260
Time*treatment	0.062	0.287	0.055	0.293	0.374	0.013	0.357

Values with different letters in the same column are significantly different ($p < 0.05$) ($n = 2$).

Table 4
Swelling capacity (S.C.), water absorption capacity (WHC), water retention capacity (WRC), and oil retention capacity (OHC) of the cake without treatment and the cake with autoclave and hot air treatments.

	SC	WHC	WRC	OHC
Control	5.094 ± 0.124^{a}	6.687 ± 0.651^{a}	4.751 ± 0.105^{a}	1.884 ± 0.110^{c}
AC 10	3.231 ± 0.189^{c}	4.824 ± 0.272^{cd}	3.456 ± 0.071^{b}	2.462 ± 0.143^{ab}
AC 20	3.921 ± 0.278^{b}	5.132 ± 0.359^{bcd}	3.129 ± 0.178^{b}	2.002 ± 0.052^{c}
AC 30	3.400 ± 0.201^{c}	4.705 ± 0.329^{d}	3.119 ± 0.101^{c}	2.002 ± 0.036^{c}
HAS 10	3.306 ± 0.245^{c}	5.732 ± 0.566^{b}	3.505 ± 0.099^{b}	2.577 ± 0.035^{a}
HAS 20	3.285 ± 0.034^{c}	5.226 ± 0.264^{bcd}	3.140 ± 0.102^{c}	2.405 ± 0.057^{b}
HAS 30	3.251 ± 0.346^{c}	5.523 ± 0.330^{bc}	3.305 ± 0.139^{bc}	2.591 ± 0.066^{a}

Data are the mean values ± S.D. of six independent experiments ($n = 6$). Different superscript letter within the column indicates significant differences ($p \leq 0.05$).

Consequently, the polypeptide chains of high molecular weight compounds in the samples with high temperature treatment associate and precipitate [60]. This means that with treatment at higher temperatures, the hydration capacity of Sacha inchi flour decreases compared to the control. This relationship is consistent with results obtained in soy flour and protein concentrates derived from soy [64].

The technofunctional properties S.C., WHC, and WRC have significant differences ($p \leq 0.05$) in relation to the control test. However, in the flour of other legumes with a lower protein content, the opposite occurs due to the gelatinization of the starch, which causes an increase in the technofunctional properties at high temperatures [59].

Meanwhile, there is an increase in OHC in the treatments and significant differences ($p \leq 0.05$) between the HA treatments, AC 10 min, and the control test due to modifications with residual starches and protein denaturation that could affect the content of non-polar amino acids and proteins that have an affinity for forming bonds with lipids [64,65].

3.4. Further research and future studies

Hydrothermal treatments are commonly used to alter the structure of starches in many cereals and pseudocereals. Research has been conducted to study the impact of factors such as the water-sample relationship, exposure time, and temperature on techno-

functional properties, digestibility, gelatinization, and other properties. Additionally, studies have demonstrated that hydrothermal treatments can also be employed to eliminate antinutrients in lentils, red kidney beans, white kidney beans, chickpeas, black grams, soybeans, and plum kernel grits [66–68].

The application of studies in which tests are carried out with treatments with different levels of humidity, time and temperature are necessary to identify the best treatment for the elimination of antinutrients and also determine the effect on the techno-functional and nutritional properties of Sacha inchi flour. Finally, the use of unconventional technologies such as ultrasound and microwaves in combination with hydrothermal treatments is desirable to improve the antinutrient removal process and provide the industry with better scalable methods.

4. Conclusion

The application of heat treatments using autoclave and hot air sterilization led to a significant reduction in several antinutrients such as alkaloids, oxalates, nitrates, tannins, saponins, phytic acid, and trypsin inhibitors and thiocyanates, wich did not show reduction. Additionally, a substanctial increase in protein content was observed in treatments conducted in autoclave and hot air for 10 min. The increase in protein content was correlated with the amino acids present, and those formed during the hydrothermal treatments were reconfigured in the synthesis of new proteins due to protein aggregation. Furthermore, the hydration properties decreased due to the denaturation of proteins during thermal treatments, thereby generating an affinity for nonpolar compounds, such as fats. This phenomenon led to increased fat retention capacity, referred to as OHC.

Finally, the current study has limitations in determining parameters like total, soluble and insoluble fiber. However, the absence of these results did not impact the discussion and explanation of the changes caused by hydrothermal treatments in the antinutrients, techno-functional properties, and nutritional properties.

CRediT authorship contribution statement

Edgar Landines Vera: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Conceptualization. **Elena Villacrés:** Writing – original draft, Validation, Resources, Investigation, Conceptualization. **Karin Coello Ojeda:** Visualization, Resources, Project administration, Investigation, Formal analysis. **Verónica Guadalupe Moyano:** Writing – original draft, Visualization, Formal analysis. **Marco Quezada Tobar:** Writing – original draft, Visualization, Conceptualization. **María Belén Quelal:** Validation, Resources, Investigation. **Yadira Quimbita Yupangui:** Validation, Resources, Investigation. **Jenny Ruales:** Writing – review & editing, Visualization, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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